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Noncovalent Interactions of Poly(adenosine diphosphate ribose) with Histones†

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ABSTRACT: Covalent linkage of ADP-ribose polymers to proteins is generally considered essential for the posttranslational modification of protein function by poly(ADP-ribosyl)ation. Here we demonstrate an alternative way by which ADP-ribose polymers may modify protein function. Using a highly stringent binding assay in combination with DNA sequencing gels, we found that ADP-ribose polymers bind noncovalently to a specific group of chromatin proteins, i.e., histones H1, H2A, H2B, H3, and H4 and protamine. This binding resisted strong acids, chaotropes, detergents, and high salt concentrations but was readily reversible by DNA. When the interactions of variously sized linear and branched polymer molecules with individual histone species were tested, the hierarchies of binding were branched polymers > long, linear polymers > short, linear polymers and H1 > H2A > H2B = H3 > H4. For histone H1, the target of polymer binding was the carboxy-terminal domain, which is also the domain most effective in inducing higher order structure of chromatin. Thus, noncovalent interactions may be involved in the modification of histone functions in chromatin.

During DNA excision repair of higher eukaryotes, large numbers of protein-bound adenosine diphosphate ribose (ADP-ribose)[1] polymers are processed by the poly(ADP-ribosyl)ation system of chromatin. The reaction cycle begins with postincisional activation of the enzyme poly(ADP-ribose) polymerase (EC 2.4.2.30), automodification of the enzyme, and modification of other DNA-binding proteins with variously sized ADP-ribose polymers. Protein-bound polymers are then rapidly degraded by the consecutive actions of poly(ADP-ribose) glycohydrolase and ADP-ribosyl protein lyase [for reviews, see Ueda and Hayaishi (1985), Ueda (1987), and Althaus and Richter (1987)].

The molecular mechanism whereby poly(ADP-ribosyl)ation primes chromatin proteins for an active role in DNA excision

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[1] Abbreviations: ADP-ribose, adenosine diphosphate ribose; AMP, adenosine 5′-monophosphate; NAD^+, β-nicotinamide adenine dinucleotide; Tris, tris(hydroxymethyl)aminoethane; EDTA, ethylenediaminetetraacetic acid; HPLC, high-performance liquid chromatography.

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repair is not understood. The prevalent view is that covalent linkage of ADP-ribose polymers is essential for the modification of target protein function. Adaptation of protein function by poly(ADP-ribose) may be mediated by a combination of factors, i.e., the numbers, sizes, and branching structures of ADP-ribose polymers as well as the choice of specific amino acid acceptor sites. These variables may themselves be tightly regulated by other nuclear factors. We recently demonstrated that histones H1, H2A, H2B, H3, and H4 specifically affect the polymer termination reaction of poly(ADP-ribose) polymerase in vitro. This led to the formation of histone type-specific polymer patterns (Naegeli & Althaus, 1991), suggesting a role for noncovalent histone–polymer interactions in the modulation of chromatin functions by poly(ADP-ribosyl)ation.

In the present study, we have set up a novel in vitro system to test for noncovalent binding of proteins to specific molecular classes of ADP-ribose polymers. Unspecific electrostatic interactions were eliminated by highly stringent assay conditions. Surprisingly, of 25 proteins tested, only histones H1, H2A, H2B, H3, and H4 and protamine bound to ADP-ribose polymers. The results show that polymers of different complexities and sizes differ with regard to protein-binding capacity. Also, the polypeptide domain involved in polymer binding of histone H1 is identical to the one that is most effective in inducing higher order chromatin structure. Thus, noncovalent interactions with ADP-ribose polymers may modulate histone functions.

Experimental Procedures

Materials. Poly(ADP-ribose) polymerase was purified from calf thymus as described by Naegeli et al. (1989). Purified poly(ADP-ribose) glycohydrolase was obtained from calf thymus using the method of Thomassin et al. (1990) except that the DNA–agarose and heparin–Sepharose column chromatography steps were omitted. Electrophoretically pure histones H1, H2A, and H2B were purchased from Boehringer-Mannheim along with proteinase K, DNA polymerase I (Klenow fragment), micrococcal nuclease, and tRNA. Histones H3 and H4 were purified from rat liver nuclei according to the procedure of Gurley et al. (1983). Electrophoretically pure fragments of histone H1 (amino terminal, globular, and carboxy terminal) were prepared as described (Naegeli & Althaus, 1991). DNA polymerases α, δ, and ϵ were purified from calf thymus and kindly provided by Dr. U. Hübscher (same institute). A histone mixture (64% H1, 36% other, by electrophoretic analysis), deoxyribonuclease I, Rec A protein, serum amyloid P component, cytochrome *c*, lysozyme, salmon sperm protamine, bovine serum albumin, calf thymus DNA, and polymeric L-aspartate, L-glutamate, L-arginine, and L-lysine were obtained from Sigma. [*adenylate*-^{32}P]NAD$^+$ (800 Ci/mmol) was purchased from New England Nuclear. T4 DNA ligase, calf thymus topoisomerase I, DNA gyrase, and ribonuclease H were from Gibco/BRL; nuclease S1 and single-stranded DNA-binding protein were from Pharmacia; heparin and sodium dodecyl sulfate were from Fluka; snake venom phosphodiesterase was from Worthington. Proteins listed in Table I were all >95% pure. All other chemicals were of the highest quality commercially available.

Synthesis and Preparation of Protein-Free Poly(ADP-ribose). Polymers of ADP-ribose were synthesized in a 100-μL reaction mixture containing 500 ng of poly(ADP-ribose) polymerase, 2.5 μg of nicked calf thymus DNA (Loeb, 1969), 150 μM [^{32}P]NAD$^+$ (1000–2000 dpm/pmol), 25 mM Tris (pH 8.0), 10 mM $MgCl_2$, 0.5 mM dithiothreitol, and 0.1 M NaCl. After a 30-min incubation at 25 °C, the reaction was stopped by addition of ice-cold trichloroacetic acid to a final concentration of 20% (w/v).

Table I: Poly(ADP-ribose) Binding Ability of Various Proteins Using the Phenol Partitioning Assay

	p*I*	binding to poly(ADPR)
histones and protamine		
H1	basic	+
H2A	basic	+
H2B	basic	+
H3	basic	+
H4	basic	+
protamine	basic	+
H1 (COOH-terminal domain)	basic	+
H1 (globular domain)	basic	−
H1 (H_2N-terminal domain)	basic	−
non-DNA-binding proteins		
bovine serum albumin	acidic	−
cytochrome *c*	basic	−
lysozyme	basic	−
proteinase K	basic	−
DNA-binding proteins (non-histone)		
poly(ADP-ribose) polymerase	basic	−
deoxyribonuclease I	acidic	−
nuclease S1	acidic	−
T4 DNA ligase	acidic	−
topoisomerase I	acidic	−
DNA gyrase	acidic	−
Pol I (Klenow fragment)	acidic	−
DNA Pol α	acidic	−
DNA Pol δ	acidic	−
DNA Pol ϵ	acidic	−
ribonuclease H	acidic	−
ssDNA-binding protein	acidic	−
micrococcal nuclease	basic	−
Rec A protein	acidic	−
serum amyloid P component	acidic	−

The protein-bound polymers were collected by centrifugation and dissolved in 150 μL of 10 mM Tris (pH 12)/1 mM EDTA. Intact polymers were detached from the protein by incubation for 3 h at 60 °C. The protein was extracted into an equal volume of phenol/$CHCl_3$/isoamyl alcohol (49:49:2) and the resulting aqueous phase dried in a Speed-Vac concentrator. The [^{32}P]poly(ADP-ribose) pellet was dissolved in water and stored at 4 °C.

Analysis of Protein Binding to Poly(ADP-ribose). The protein to be tested was incubated with [^{32}P]poly(ADP-ribose) in 20 μL of 10 mM Tris (pH 7.0)/1 mM EDTA for 10 min at 25 °C. The sample was diluted to 100 μL with the same buffer and extracted with phenol/$CHCl_3$/isoamyl alcohol as above. To the aqueous phase were added 12.5 μL of 3 M sodium acetate, 3 μL of tRNA (15 μg), and 375 μL of cold 96% ethanol. After precipitation at −20 °C for 4 h, [^{32}P]-poly(ADP-ribose) was pelleted by centrifugation (14750*g*, 4 °C, 30 min), dried in a Speed-Vac, and immediately subjected to size analysis on high-resolution DNA sequencing gels. Recovery of polymers from the phenol phase in the absence of polymer-binding protein was always >95%. In the presence of polymer-binding protein, control experiments showed that the bound polymer fraction exactly complemented the unbound fraction. Unless otherwise noted, only the unbound polymer fractions are presented. Results varied <2% between experiments using the same polymer population and unthawed histone preparation.

Size Analysis of Poly(ADP-ribose) Using High-Resolution DNA Sequencing Gels. High-resolution size analysis of ^{32}P-labeled polymers was performed as described (Panzeter & Althaus, 1990). Briefly, polymers were dissolved in 10 μL of loading buffer and separated on a 20% polyacrylamide gel in

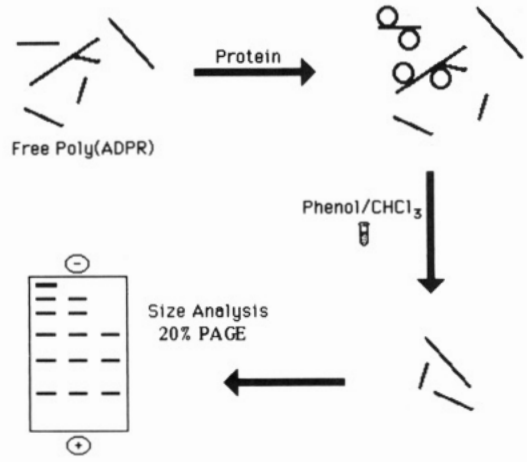


FIGURE 1: Phenol partitioning assay. Schematic diagram showing the experimental approach. A given protein is incubated in a solution of free poly(ADP-ribose) and subsequently extracted with phenol/$CHCl_3$/isoamyl alcohol. If the protein–poly(ADP-ribose) interaction is sufficiently strong to withstand extraction, the protein-bound polymer enters the organic phase. Polymers remaining in the aqueous phase are collected and analyzed by polyacrylamide gel electrophoresis. Polymers in the organic phase can also be recovered and analyzed (e.g., Figure 5). In this way, the type of polymers to which the protein binds can be determined. A more detailed explanation is provided under Experimental Procedures. ADPR = ADP-ribose.

0.09 M Tris, 0.09 M borate, and 2 mM EDTA at 55 W (constant power). The gel was dried and subjected to autoradiography. Unless otherwise noted, the gels shown are representative results of at least three experiments.

HPLC Analysis of Poly(ADP-ribose). The composite structure of poly(ADP-ribose) was determined using strong anion-exchange HPLC as described (Alvarez-Gonzalez & Jacobson, 1987). Briefly, ^{32}P-labeled polymers were digested with snake venom phosphodiesterase to give AMP, phosphoribosyl-AMP (PRAMP), and di(phosphoribosyl)-AMP [di(PR)AMP]. These were separated isocratically using 125 mM KH_2PO_4 (pH 4.7)/0.5 M KCl at 1 mL/min. Average polymer size, chain length, and branching frequency were calculated using the equations:

average polymer size:

$$\frac{[\text{AMP}] + [\text{PRAMP}] + [\text{di(PR)AMP}]}{[\text{AMP}] - [\text{di(PR)AMP}]}$$

average chain length:

$$\frac{[\text{PRAMP}]}{[\text{AMP}]} + 1$$

average number of branches per polymer:

$$\frac{[\text{di(PR)AMP}]}{[\text{AMP}] - [\text{di(PR)AMP}]}$$

Results

Noncovalent Binding of Histones to Poly(ADP-ribose). We have set up an in vitro test system for the identification of noncovalent protein–poly(ADP-ribose) interactions under conditions of high stringency. Figure 1 outlines the experimental approach. The test protein was incubated with a mixture of ^{32}P-labeled ADP-ribose polymers containing both linear and branched molecules. The reaction mixture was extracted with phenol/chloroform/isoamyl alcohol to separate protein–polymer complexes from unbound ADP-ribose polymers. The unbound polymers from the aqueous phase were loaded onto modified DNA sequencing gels (Panzeter & Althaus, 1990) and separated according to molecular size.

The application of this technique to a histone mixture and purified histone H1 is shown in Figure 2. Histones changed

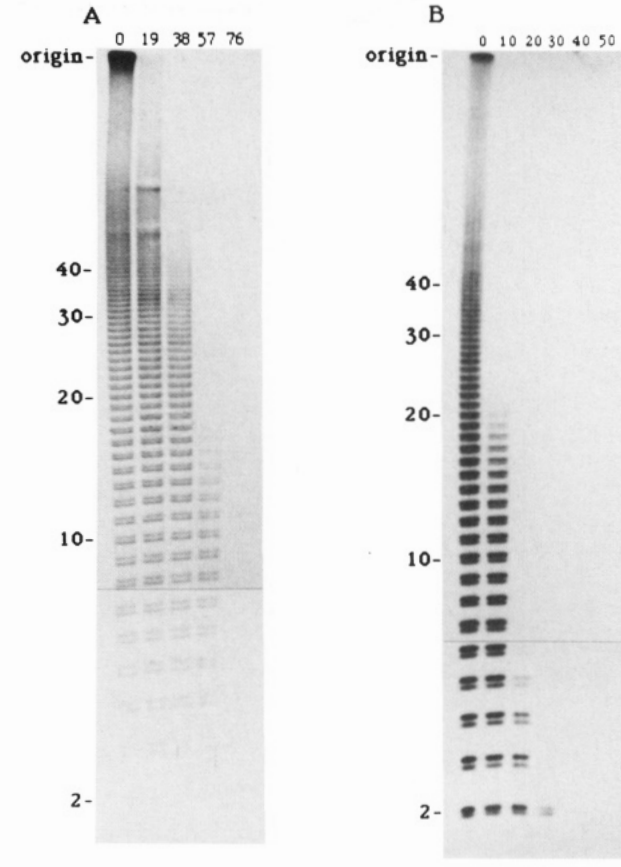


FIGURE 2: Histone binding to free poly(ADP-ribose). The indicated picomoles of a histone mixture (A) and electrophoretically pure H1 (b) were added to 93 and 147 pmol, respectively, of ADP-ribose in the form of polymers. Protein binding to poly(ADP-ribose) was analyzed by the phenol partitioning assay. The lengths of the polymers in terms of ADP-ribose residues are indicated on the left.

the phenol partitioning of ADP-ribose polymers in a dose-dependent manner (Figure 2A). Interestingly, the unresolved polymers at the top of the gel were most susceptible to histone binding, while the reactivity of polymers capable of entering the gel decreased gradually with polymer size. Purified histone H1 reacted more effectively with ADP-ribose polymers (Figure 2B), but a preference for the top-of-the-gel fraction and larger polymer sizes was again observed. Quantitative analysis (Figure 3A) showed a plateau with more than 95% of all ADP-ribose residues bound to histone. The preference of H1 binding to different polymer size classes was calculated, and the results are shown in Figure 3B. When relatively small amounts (10 pmol) of histone H1 were added to the polymer mixture, up to 7.8 ADP-ribose residues were coextracted with each molecule of H1. Comparison of Figures 3B and 2B reveals that these polymers belonged to the top-of-the-gel fraction and the larger polymer size classes. At the other extreme, only three ADP-ribose residues were extracted per histone molecule when the highest amount of H1 (50 pmol) was added. The smaller sized ADP-ribose oligomers, which were free in solution at low histone concentration, became thus associated with histone (Figure 2B).

In the experiment shown in Figure 4, purified histones H1, H2A, H2B, H3, and H4 were tested individually for polymer binding. All histone species bound to ADP-ribose polymers, and all of them exhibited preferential binding to the polymer fraction at the gel origin. The molecules of ADP-ribose, in the form of radiolabeled polymers, partitioned into the organic phase as a result of histone binding were quantitated, and the average binding capacity for each histone species was estab-

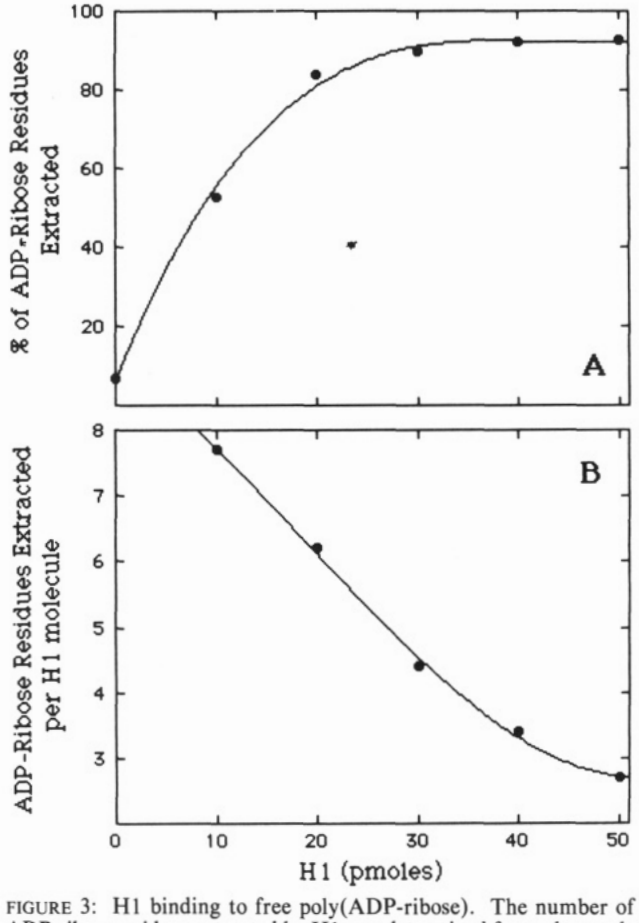


FIGURE 3: H1 binding to free poly(ADP-ribose). The number of ADP-ribose residues extracted by H1 was determined for each sample in Figure 2B. H1 binding to polymers of ADP-ribose was completely saturable (A). However, the number of ADP-ribose residues extracted per histone molecule was inversely dependent on the amount of H1 added (B).

lished as the ratio of ADP-ribose residues bound per histone molecule: 7.5 for histone H1, 5 for histone H2A, 4 for histone H2B, 4 for histone H3, and 2.6 for histone H4.

Stability of Histone–Polymer Complexes. Histone–polymer complexes exhibited a remarkable resistance to treatment with acids, high salt concentrations, detergents, and chaotropes. In fact, histones remained bound to ADP-ribose polymers in the presence of 0.2 M HCl, 0.2 M H_2SO_4, 1 M acetic acid, 1 M NaCl, 0.25% Triton X-100, 4 M urea, or sodium dodecyl sulfate concentrations below 2%. These conditions have previously been used to isolate and analyze histones covalently modified with ADP-ribose polymers. However, histone–polymer complexes could be readily dissolved by 2% sodium dodecyl sulfate or double-stranded DNA. Figure 5 shows the displacement of different polymer fractions from histone H1 by DNA. Short polymers were first displaced, and as more, competing DNA was added, larger polymers were released from H1. Thus, as expected, the order of complex dissociation was exactly the reverse of complex formation. In addition, since 70% of total ADP-ribose was rendered phenol-extractable by H1 (Figure 5, second lane), and 95% of these ADP-ribose polymers were released at a nucleotide:ADP-ribose ratio of 0.70 (Figure 5, fifth lane), these experiments revealed that one nucleotide of DNA (having one negative charge) effectively competed with the binding of one ADP-ribose residue (having two negative charges). The same phenomenon was observed with all other histone species (not shown). Neither heparin nor tRNA, added to the same residue concentration as DNA, had an effect on histone binding to polymers.

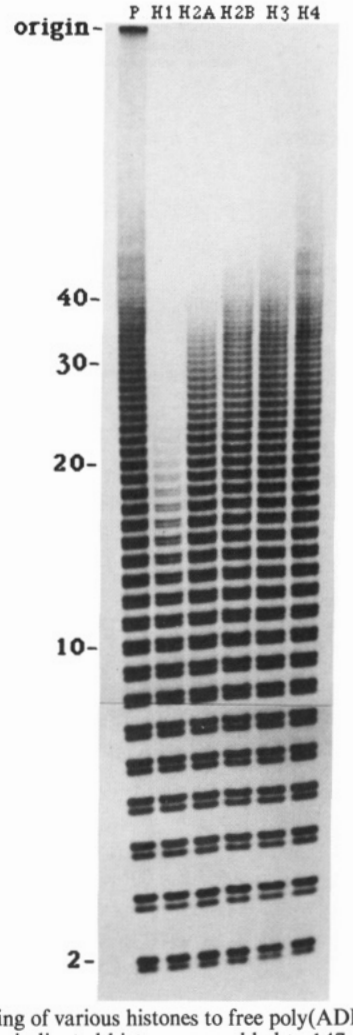


FIGURE 4: Binding of various histones to free poly(ADP-ribose). Ten picomoles of the indicated histone was added to 147 pmol of ADP-ribose in the form of polymers. Protein binding to poly(ADP-ribose) was analyzed by the phenol partitioning assay. The first lane, P, represents polymers to which no protein was added. The lengths of the polymers in terms of ADP-ribose residues are indicated on the left.

Polymer Fraction at the Gel Origin Contains Branched Polymers. Since all histone species tested exhibited preferential binding to the polymer fraction at the gel origin, it was of considerable interest to determine whether this polymer fraction represents poly(ADP-ribose) with unique structural features or simply polymers of extreme length. Therefore, the phosphodiesterase degradation products of these polymers were analyzed by strong anion-exchange chromatography to determine the average polymer size as well as the average branching frequency (Alvarez-Gonzalez & Jacobson, 1987). Interestingly, the average size of polymers remaining at the gel origin was only 54.9 (Figure 6, lane C), a size which should readily migrate into the gel (Panzeter & Althaus, 1990). However, this fraction contained a significant proportion of branched residues, whereas the polymers migrating into the gel were devoid of branches (Figure 6, lane B). This suggested that the presence of branched residues prevents polymers from entering this type of gel and that the highest preference of histone binding was to branched ADP-ribose polymers.

Branched ADP-ribose Polymers Bound to Histones Are Protected from Poly(ADP-ribose) Glycohydrolase Digestion. When a mixture of protein-free branched and linear ADP-ribose polymers was incubated with the enzyme poly(ADP-ribose) glycohydrolase, polymer digestion to ADP-ribose was complete by 60 min (Figure 7, lanes "–H2B"). It was noted

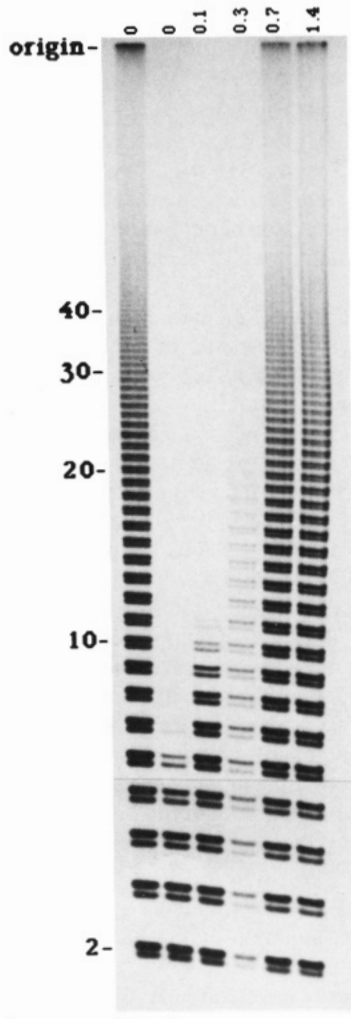
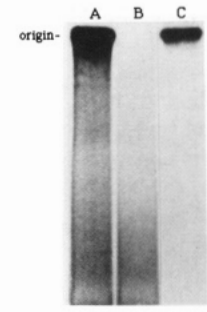


FIGURE 5: Displacement of H1 from poly(ADP-ribose) by DNA. Thirty-eight picomoles of H1 was incubated for 10 min at 25 °C with 212 pmol of ADP-ribose in the form of polymers. Increasing amounts of a 236 bp DNA fragment (10 mg/mL) were added and the samples incubated as above. Protein binding to poly(ADP-ribose) was analyzed by the phenol partitioning assay. (Only 35% of the total sample was loaded onto the gel in lane four.) The numbers above each lane indicate the picomoles of nucleotides added per picomole of ADP-ribose. No histones were added to the polymers in the first lane. The lengths of the polymers in terms of ADP-ribose residues are indicated on the left.



	Average Polymer Size	Average Chain Length	Branches per Polymer
A	20.4 ± 0.7	14.8 ± 0.4	0.36 ± 0.02
B	12.8 ± 1.6	12.6 ± 0.8	0.00 ± 0.02
C	54.9 ± 3.3	22.9 ± 1.1	1.33 ± 0.03

FIGURE 6: Characterization of poly(ADP-ribose) at the gel origin. Enough H1 was incubated with poly(ADP-ribose) to result in the phenol extraction of only those polymers which remain at the gel origin (see Figure 2A). The aqueous phase—containing the polymers which enter the gel—was collected. More than 95% of the polymers in the phenol phase were recovered upon extraction with 0.1 mg/mL DNA which competes for histone binding, thereby releasing the polymers (see Figure 5). Total poly(ADP-ribose), (A), poly(ADP-ribose) which enters the gel (B), and poly(ADP-ribose) from the gel origin (C), were subjected to polyacrylamide gel electrophoresis and HPLC analyses as described under Experimental Procedures (N = 2).

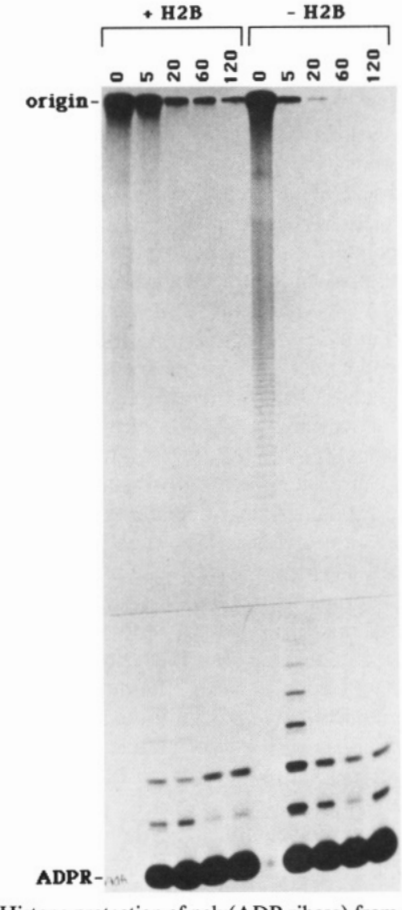


FIGURE 7: Histone protection of poly(ADP-ribose) from glycohydrolase digestion. 120 pmol of ADP-ribose in the form of protein-free polymers was incubated with 0.9 ng (5.5 milliunits) of poly(ADP-ribose) glycohydrolase in the absence or presence of 6.5 pmol of H2B. The reaction buffer included 8 mM Tris (pH 8.0), 80 mM NAD^+, 60 ng of 146 bp core DNA, and 10 ng of poly(ADP-ribose) polymerase inhibited by 6.5 mM benzamide. The times of incubation (minutes) are indicated above each lane. Time 0 represents polymers incubated for 120 min in the presence of heat-inactivated glycohydrolase. Digestions were terminated by heating at 60 °C for 5 min. After proteinase K digestion of protein followed by phenol extraction, polymers were subjected to polyacrylamide gel electrophoresis as described under Experimental Procedures. ADPR = ADP-ribose.

that linear polymers were degraded slightly more rapidly than branched polymers. However, in the presence of histones, overall degradation of polymers was much slower. Figure 7 (lanes "+H2B") illustrates the protective effect of histone H2B on the degradation of a mixture of branched and linear polymers. While subfractions of linear polymers were degraded at the same rate in the absence or presence of H2B, polymers at the gel origin, i.e., branched polymers, resisted degradation by poly(ADP-ribose) glycohydrolase, even after an incubation time of 120 min.

Specificity of Protein Binding by Poly(ADP-ribose). Poly(ADP-ribose) contains two negative charges per nucleotide and is more acidic than DNA. In the experiments shown in Table I, we tested whether binding to poly(ADP-ribose) is a general property of basic and/or DNA-binding proteins. The results showed that only protamine, which replaces histones in the final stages of spermatogenesis (Risley et al., 1986), bound to poly(ADP-ribose) under the highly stringent conditions described in Figure 1. All other cellular proteins tested at equimolar concentrations were found negative in this assay. As expected from results by Naegeli and Althaus (1991), synthetic homopolymers of L-lysine and L-arginine also bound to polymers of ADP-ribose, whereas polymers of L-aspartate and L-glutamate did not.

To further elucidate the polypeptide domains involved in polymer binding, histone H1 was digested into three polypeptides representing the amino-terminal, central-globular, and carboxy-terminal domains of H1 (Naegeli & Althaus, 1991). The carboxy-terminal fragment exhibited the same binding capacity as intact H1, while the other fragments were negative in this assay (Table I). This finding raised the possibility that histones bind to poly(ADP-ribose) via a basic, α-helical motif (Turnell et al., 1988; Churchill & Travers, 1991). Such a protein motif exists in the carboxy-terminal domain of H1 and mediates H1 binding to DNA (Hill et al., 1989). Rec A protein also possesses this motif but failed to bind to poly(ADP-ribose). In addition, serum amyloid P component, a member of the pentraxin family of plasma proteins containing the same motif, has been shown to bind to chromatin by displacing histone H1 (Butler et al., 1990) but also failed to bind to ADP-ribose polymers (Table I). To date, cellular proteins binding to poly(ADP-ribose) are confined to histones and protamine, underlining the specificity of this interaction.

Discussion

The covalent bond between ADP-ribose polymers and their acceptor proteins has been considered essential for the modification of protein function by poly(ADP-ribosyl)ation [e.g., see de Murcia et al. (1986, 1988)]. However, unlike most other posttranslational protein modifications, the modifying residues are variously sized and structured macromolecules which may add several hundred thousand daltons of molecular mass to a protein. An example is the nuclear enzyme poly(ADP-ribose) polymerase, which may carry up to 22 polymers and which is the predominant acceptor protein in mammalian cells during DNA excision repair [for reviews, see Althaus and Richter (1987) and de Murcia et al. (1988)]. Histones regulate the sizes and complexities of polymerase-bound polymers (Naegeli & Althaus, 1991). Furthermore, polymerase-bound polymers can induce the dissociation of histones from DNA templates (Mathis & Althaus, 1987; Althaus et al., 1989; Realini, 1991). It was therefore of considerable interest to determine whether noncovalent polymer–protein interactions do indeed occur and to see whether such interactions would exhibit any specificity with regard to the macromolecules and macromolecular domains involved.

In designing an in vitro system to study noncovalent polymer–protein interactions, the following criteria had to be met: (i) highly stringent conditions to eliminate unspecific electrostatic interactions; (ii) the possibility to study and quantify the interactions of specific polymer molecules with specific protein species; (iii) the possibility to identify the sites of macromolecular interactions. For this purpose, a phenol partitioning assay was combined with high-resolution gel electrophoresis. The results show that ADP-ribose polymers bound selectively to a specific group of chromatin proteins, i.e., histones H1, H2A, H2B, H3, and H4 and protamine. That this binding was not a general property of basic and/or DNA-binding proteins was shown by the results in Table I. The strength of binding was unexpectedly high as illustrated by the resistance of the polymer–protein complexes to phenol partitioning, high salt concentrations, detergent, chaotropes, and strong acids. Examining the interactions of different histone types with specific polymer molecules revealed an additional level of specificity: the most highly preferred binding counterparts were branched ADP-ribose polymers followed by long and short linear polymers, respectively. Most interestingly, only one proteolytic fragment of histone H1 was a target of polymer binding, i.e., the α-helical, basic (40 mol % lysine), carboxy-terminal domain which binds tightly to DNA and is the most effective portion of H1 in inducing higher order chromatin structures in vitro (Bradbury et al., 1975; Thoma et al., 1983).

Previous reports by others have shown that poly(ADP-ribosyl)ation may modulate chromatin structure [for a review, see de Murcia et al. (1988)]. For example, poly(ADP-ribosyl)ation of chromatin preparations of various complexities induced decondensation of chromatin superstructure as shown by electron microscopy and analytical ultracentrifugation (Poirier et al., 1982; de Murcia et al., 1986, 1988). This has been attributed to the covalent modification of histone H1 (de Murcia et al., 1988) which, in our study, was the histone species binding most strongly to ADP-ribose polymers. However, our results show that polymers noncovalently bound to histone H1 become phenol-extractable and are resistant to high salt concentrations, chaotropes, detergents including 1% sodium dodecyl sulfate, and strong acids, i.e., conditions which have been used routinely in the isolation of covalently modified and unmodified histones. Thus, we propose that noncovalent interactions of ADP-ribose polymers with histones may be important in modulating chromatin structure by poly(ADP-ribosyl)ation. This view is supported by the fact that automodified poly(ADP-ribose) polymerase can dissociate DNA–histone complexes in the absence of covalent histone modification (Althaus et al., 1989; Realini, 1991). Nevertheless, covalent histone modifications by poly(ADP-ribose) polymerase may play an important role in destabilizing intranucleosomal DNA–protein interactions as has been shown for acetylation (Oliva et al., 1990) and ubiquitination (Davie & Murphy, 1990).

Although all histones bound to poly(ADP-ribose), they differed in their molar capacities for binding, the order being H1 > H2A > H2B = H3 > H4. Assuming that histones in chromatosomes behave similarly to those in solution when presented with an ADP-ribose polymer, the results in this study show that 1 polymer molecule of about 40 residues would be sufficient to bind the 9 histone molecules of a single chromatosome. The differential binding capacities could reflect a mechanism by which the spatial and/or temporal sequence of histone binding is determined. Evidence in support of this concept is derived from the fact that histones induce the polymerase to produce histone type-specific polymer patterns and the polymer pattern found in mammalian cell nuclei was indistinguishable from the composite pattern induced by the histones of a chromatosome (Naegeli & Althaus, 1991). Thus, the strong and differential binding of histones to ADP-ribose polymers (this report) correlates with their potential to induce a specific pattern of polymers. Conversely, proteins that did not show binding to polymers failed to regulate the polymer size pattern produced by poly(ADP-ribose) polymerase.

In view of the available literature, the present results may reflect a biological role of the poly(ADP-ribosyl)ation system different from that of other posttranslational protein modifications. The key enzyme poly(ADP-ribose) polymerase may be envisioned as a protein targetable to sites of single- and/or double-strand DNA breaks. Two zinc fingers in the DNA-binding domain determine this target specificity (Gradwohl et al., 1990; Ikejima et al., 1990). Upon activation by single- or double-strand DNA breaks, the polymerase converts into a protein carrying up to 22 polymers per enzyme molecule. We have already shown that these polymerase-bound polymers can effectively dissociate DNA–histone complexes (Althaus et al., 1989; Realini, 1991). This paper reports the noncovalent but specific binding of histones to these polymers. Thus, the poly(ADP-ribosyl)ation system may be viewed as an en-

zyme-catalyzed, histone-scavenging mechanism. Preliminary data from our laboratory indicate that this mechanism may establish accessibility of DNA domains to other proteins (Realini and Althaus, unpublished experiments).

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